The Potent Inhibitory Effect of a Naproxen-Appended Cobalt(III)-Cyclam Complex on Cancer Stem Cells

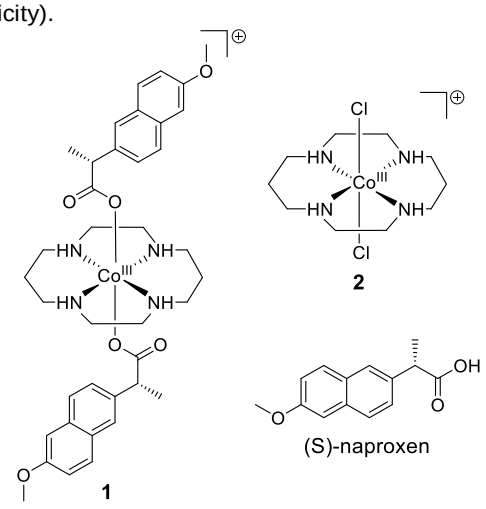
Paul B. Cressey, Arvin Eskandari, Peter M. Bruno, Chunxin Lu, Michael T. Hemann, and Kogularamanan Suntharalingam*

Abstract: We report the cancer stem cell (CSC) potency of a novel cobalt(III)-cyclam complex, **1** bearing the nonsteroidal anti-inflammatory drug, naproxen. The cobalt(III)-cyclam complex, **1** displays selective potency for breast CSC-enriched HMLER-shEcad cells over breast CSC-depleted HMLER cells. Additionally, **1** inhibits the formation of three-dimensional tumor-like mammospheres, and reduces their viability to a greater extent than clinically used breast cancer drugs; vinorelbine, cisplatin, and paclitaxel. The mammosphere-potency of **1** was enhanced in hypoxia-mimicking conditions. Detailed mechanistic studies revealed that DNA damage and cyclooxygenase-2 (COX-2) inhibition contribute to the cytotoxic mechanism of action of **1**. To the best of our knowledge, **1** is the first cobalt-containing compound to show selective potency for CSCs over bulk cancer cells.

There is mounting evidence linking fatal incidences of cancer relapse with cancer stem cells (CSCs), a subpopulation of cancer cells with the ability to self-renew, differentiate, and initiate tumor growth.[1] Due to their remarkable stem cell-like properties, CSCs are thought to be key drivers in tumor progression and therapeutic resistance.[2] Surgery in combination with chemotherapy and radiotherapy effectively reduces tumor mass by removing non-tumorigenic, bulk cancer cells, but they are unable to eliminate CSCs.[3] CSCs remain largely untouched by current therapeutic regimens, and can initiate tumor regrowth. Therefore to improve clinical outcomes, treatments must have the ability to kill all forms of cancer cells, including CSCs. Although several kinks in the CSC armoury have been identified, such as deregulated signalling pathways, overactive organelles, and cell surface markers,[4] there is still no clinically approved drug (chemical or biological) that can selectively remove CSCs.

Cytotoxic compounds capable of accumulating and undergoing activation in microenvironments (niches) supporting CSCs can potentially kill CSCs specifically.[4a,5] The CSC microenvironment is thought to be hypoxic, and a clear link between hypoxia inducible factors (HIFs), particularly HIF1α and HIF2α, and CSC regulation is now emerging.[6] Recent studies indicate that HIF1α and HIF2α are highly expressed in breast, glioma, and neuroblastoma CSCs, and play pivotal roles in CSC self-renewal.[7] Here, we present a cobalt(III)-cyclam complex capable of releasing naproxen, a nonsteroidal anti-inflammatory drug (NSAID), under reducing conditions. Naproxen is an inhibitor of cyclooxygenase isoenzymes, COX-1 and COX-2.[8] Cyclooxygenases catalyse the biosynthesis of prostaglandins (PGs), which are mediators in inflammatory reactions. The inducible isoform, COX-2 is highly expressed in certain CSCs and promotes stem cell renewal, proliferation, and radioresistance.[9] Therefore, inhibition of COX-2 using small molecules like NSAIDs provides an effective method of sensitizing CSCs to cytotoxic agents. We and others have recently shown that free NSAIDs and metal-NSAIDs conjugates can supress CSC proliferation.[10]

Cobalt is an essential trace element found in all animals and plays a crucial role in several biologically important processes.[11] Over the last three decades, several cobalt-containing compounds have been investigated for their anticancer activity.[12] The difference in reactivity of the accessible oxidation states of cobalt(II and III) has enabled the development of cobalt(III) prodrugs that can undergo bioreductive activation in hypoxic regions. Several cobalt(III) complexes with tetradentate ligands (such as 1,4,7,10-tetraazacyclododecane, 1,4,8,11-tetraazacyclotetradecane, and tris(2-pyridylmethyl)amine) have been used to deliver therapeutic and imaging agents to hypoxic tumor microenvironments.[13] Despite this there have been no reports on the anti-CSC properties of such cobalt-containing compounds. Here, we combine naproxen with cobalt(III)-cyclam (where cyclam = 1,4,8,11-tetraazacyclotetradecane) in an attempt to release naproxen in CSCs. The role of naproxen is two-fold; it inhibits COX-2, which is overexpressed in CSCs, and aids cell uptake of the cobalt moiety (due to its inherent lipophilicity).



Scheme 1. Chemical structures of the compounds (**1**, **2**, and naproxen) under investigation.

The chemical structure of the new cobalt(III)-cyclam complex, **1** investigated in this study is depicted in Scheme 1. The cobalt(III)-cyclam complex, **1** was prepared by reacting *trans*-dichloro(cyclam)-cobalt(III) chloride, **2** with 2.5 equivalents of naproxen in methanol (dried over Na_2SO_4), in the presence of silver(I) oxide. The cobalt(III)-cyclam complex, **1** was isolated as a purple hexafluorophosphate salt and characterized by ^1H and ^{13}C

[*] Dr K. Suntharalingam, Dr. C. Lu, Mr. P.B. Cressey, Mr. A. Eskandari
Department of Chemistry
King's College London
London
SE1 1DB, UK
E-mail: kogularamanan.suntharalingam@kcl.ac.uk

Dr M. T. Hemann, Dr P. M. Bruno
The Koch Institute for Integrative Cancer Research
Massachusetts Institute of Technology
Cambridge
Massachusetts
02139, United States

Supporting information for this article is given via a link at the end of the document.

NMR, infra-red spectroscopy, mass spectrometry, and elemental analysis (see SI, Figure S1-2). Single crystals of **1** suitable for X-ray diffraction studies were obtained by layering a DMSO solution of **1** with acetone. The X-ray structure of **1** is reported in the SI (Figure S3 and Table S1-2, CCDC 148346).

As cobalt complexes containing tetradentate ligands have been widely reported to cleave DNA,[14] the DNA nuclease activity of **1** was investigated using agarose-gel electrophoresis. Upon incubation of supercoiled plasmid pUC19 DNA (100 ng) with **1** (0 - 15 μM in the absence of external reducing agents) for 16 h, a marked increase in the amount of nicked circular and linear DNA was observed (Figure S4A). Complete conversion of supercoiled to nicked circular and linear DNA was observed at 2 μM. The cobalt(III)-cyclam complex, **2** also cleaved DNA but to a lesser extent than **1** (Figure S4B). Naproxen (0 - 50 μM) did not cleave DNA under these conditions (Figure S5). In the presence of ascorbic acid, a cellular reductant, the cleavage activity of **1** increased dramatically (Figure S6A), suggestive of a redox-dependent mechanism of DNA cleavage. In contrast, **2**-induced DNA cleavage was only marginally enhanced in the presence of ascorbic acid (Figure S6B). In the presence of glutathione (10-fold excess) **1**-mediated DNA cleavage was inhibited to some extent (Figure S6C). This suggests that the DNA cleavage activity of **1** in cell-free systems is compromised by glutathione. To determine the oxidative mechanism by which **1** induces DNA cleavage, nuclease activity was probed in the presence of reactive oxygen species (ROS) scavengers (DMSO, ^{t}BuOH, KI, and NaN_3) (Figure S7A). KI and NaN_3 blocked **1**-induced DNA cleavage suggesting that hydrogen peroxide and singlet oxygen are involved in the DNA cleavage process. In the absence of oxygen, **1**-mediated DNA cleavage was blocked, reinforcing the notion of an oxidative mechanism (Figure S7B). We propose that **1** undergoes reduction to the corresponding cobalt(II) form by guanine bases in pUC19 DNA. The cobalt(II) form then reduces molecular oxygen (in solution) to superoxide, which generates hydrogen peroxide. The paramagnetic cobalt(II) form and the naproxen moiety could be responsible for singlet oxygen generation, via a photo-redox pathway in ambient light.[15] Cobalt complexes are also known to cleave DNA via hydrolytic pathways,[14a,14b] therefore the ability of **1** to induce DNA hydrolysis was probed using the T4 DNA ligase assay (see SI). pUC19 DNA that had been nicked by **1** was re-ligated by T4 DNA ligase (Figure S8), implying that **1** is able cleave DNA via a hydrolytic mechanism. We proposes that the hydrolytic mechanism involves the reduction of **1** to the corresponding cobalt(II) form followed by aquation. The aquated cobalt species could then interact with the phosphate backbone of DNA, and facilitate hydrolytic cleavage of the P–O bond.[14b,15a] Collectively, the agarose-gel electrophoresis data shows that **1** is able to cleave DNA through, both oxidative and hydrolytic pathways. However given our cleavage studies in the absence of oxygen, we should point out that the oxidative mechanism appears to play a more important role in **1**-induced DNA cleavage than the hydrolytic mechanism.

UV-Vis spectroscopy and high-resolution ESI-TOF mass spectrometry studies were carried out to assess the stability of **1** in biologically relevant solutions. In PBS and 0.9% NaCl (saline) solution, **1** is reasonably stable over a period of 24 h at 37 ^{o}C (Figure S9-10). In Tris-HCl (pH 7.4) buffer and 0.9% NaCl solution with a 10-fold excess of ascorbic acid a marked decrease in the absorption of **1** was observed over a period of 24 h at 37 ^{o}C (Figure S11-12). In 0.9% NaCl solution, significant changes to the low wavelength bands (< 300 nm) were observed, yielding a UV-Vis trace reminiscent of free naproxen (Figure S13). ESI-TOF mass spectroscopy studies of **1** in 0.9% NaCl solution with 10 equivalents of ascorbic acid after 72 h incubation, revealed a peak corresponding to $[naproxen\text{-}H+K]^-$ (268.7 *m/z*) in the negative mode (Figure S14). This shows that naproxen is liberated under reducing conditions, presumably via the reduction of the cobalt metal centre from Co(III) to Co(II). Reduction to Co(II) is known to promote total ligand substitution,[13k] which was evidenced by the appearance of new peaks corresponding to $[cyclam]^+$ (199.9904 *m/z*) and $[cyclam+K]^+$ (238.9743 *m/z*) in the positive mode of the ESI-TOF mass spectrum (Figure S15A-B). Peaks associated to cobalt(II)-cyclam species were also observed in the positive mode (Figure S15B). The lipophilicity of **1** was determined by measuring the extent to which it partitioned between octanol and water, P. The experimentally determined log P value for **1** was 0.67, indicative of hydrophobicity. The hydrophobic character of **1** suggests that the complex should be readily internalised by cells. Prior to preforming cellular studies, the stability of **1** in mammary epithelial cell growth medium (MEGM) at 37 ^{o}C was established (Figure S16).

The CSC potency and selectivity of **1**, **2**, and naproxen was investigated using two human mammary epithelial cell lines, more specifically those immortalized and transformed by retroviral expression of SV40 large T oncogene, hTERT and H-rasV12 (HMLER cells), and those subsequently subject to E-cadherin silencing by short hairpin RNA interference (HMLER-shEcad cells).[16] HMLER cells express a stable CSC-like population of 5-8%, whereas HMLER-shEcad cells express a stable CSC-like population of *ca.* 90%.[16] The cytotoxicity was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The IC_{50} values (concentration required to reduce cell viability by 50%) were calculated from dose-response curves (Figure S17-18) and are shown in Table 1. Salinomycin, a breast CSC-specific compound identified from a high-through-put screen involving 16,000 compounds, was used as a positive control (Figure S19). The cobalt(III)-cyclam complex, **1** exhibited greater potency (4-fold lower IC_{50} value, $p < 0.05$, n = 18, *statistically significant*) for CSC-enriched HMLER-shEcad cells than CSC-depleted HMLER cells. Notably, the IC_{50} value of **1** towards HMLER-shEcad cells was 46-fold lower than that of salinomycin. The potency and selectivity of **1** towards HMLER-shEcad cells was better than that of a recently reported series of copper(II)-phenanthroline complexes containing indomethacin.[10a] Naproxen and **2** were relatively non-toxic (IC_{50} > 100 μM) towards both cell lines. The discrepancy in cytotoxicity of **1** and its components, naproxen and **2**, might be due to a difference in cell uptake. To support this suggestion, cellular uptake studies were performed to investigate the CSC permeability and intracellular localisation of **1** and **2**. HMLER-shEcad cells were incubated with **1** and **2** (0.5 μM for 12 h) and the cobalt content was determined in the nuclear, cytoplasmic, membrane, and whole cell fractions using inductively coupled plasma mass spectrometry (ICP-MS) (Figure 1). The whole cell uptake of **1** (321.1 ± 10.0 ppb of Co/ million cells) was 24-fold greater than **2** (13.2 ± 0.2 ppb of Co/ million cells). Therefore the variance in cytotoxicity of **1** and **2** could indeed be related to their contrasting cellular uptake. An appreciable amount of internalised **1** was detected in the nucleus (42.0 ± 0.4 ppb of Co/ million cells). Relatively little **2** was able to penetrate the nucleus (3.3 ±

0.1 ppb of Co/ million cells). This is consistent with presence of naproxen moieties in **1** and not in **2**, as naproxen is well-known to target and inhibit COX-2 which is concentrated on the nuclear envelope.[17]

Table 1. IC_{50} values of the **1** and salinomycin against HMLER cells, HMLER-shEcad cells HMLER-shEcad mammospheres.

Compound	HMLER IC_{50} [μM][a]	HMLER-shEcad IC_{50} [μM][a]	Mammosphere IC_{50} [μM][b]
1	0.43 ± 0.05	0.11 ± 0.03	0.98 ± 0.02
salinomycin	12.17 ± 3.16	5.06 ± 1.45	14.05 ± 1.58

[a] Determined after 72 h incubation (mean of three independent experiments ± SD). [b] Determined after 120 h incubation (mean of three independent experiments ± SD).

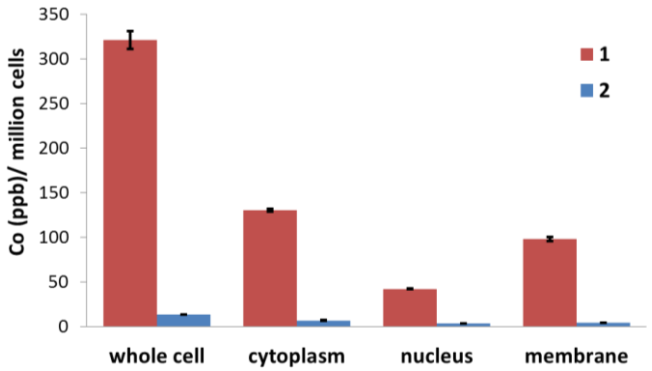


Figure 1. Cobalt content in whole cell, cytoplasm, nucleus, and membrane fractions isolated from HMLER-shEcad cells treated with **1** and **2** (0.5 μM for 12 h).

In non-adherent, serum-free cell cultures, breast CSCs can form three-dimensional tumor-like structures called mammospheres.[18] The ability of a given compound to inhibit the formation of mammospheres from single cell suspensions is a good indicator of CSC potency. Single cell suspensions of CSC-enriched HMLER-shEcad cells were treated with **1**, **2**, naproxen, and salinomycin (at their respective IC_{20} values, 5 days) and their ability to form mammospheres was determined using an inverted microscope. Upon incubation with **1**, the number of mammospheres formed decreased markedly (65% reduction) compared to the untreated control ($p < 0.01$, Figure 2A). Notably, **1**-treatment reduced the number of mammospheres formed to a greater extent than salinomycin (16% better reduction). The size of the mammospheres formed after dosage with **1** also decreased (Figure 2B). A similar decrease in mammosphere size was observed for salinomycin (Figure 2B). Naproxen- and **2**-treatment did not significantly affect the number or size of mammospheres formed (Figure 2A and S20). In order to determine the ability of **1**, **2**, naproxen, and salinomycin to reduce mammosphere viability, the colorimetric resazurin-based reagent, TOX8 was used (Figure S21 and Table S3). Identical studies were also performed with clinically used breast cancer drugs; vinorelbine, cisplatin, and paclitaxel. It should be noted that cisplatin is usually used to treat specific types of breast cancer such as triple negative or BRAC gene-related breast cancer as part of a clinical trial.[19] The IC_{50} value (concentration required to reduce mammosphere viability by 50%) of **1** was in the micromolar range. Strikingly, **1** exhibited better mammosphere-potency than salinomycin (14-fold), vinorelbine (23-fold), and cisplatin (8-fold). Naproxen and paclitaxel did not display any observable mammosphere-potency (IC_{50} > 66 μM). The latter is consistent with a previously reported study which showed paclitaxel to stimulate, rather than inhibit, mammosphere formation.[16] A recent study showed that mammosphere growth (from single cell suspensions of HMLER cells) is promoted by hypoxia, in a HIF1α-dependent manner.[20] The potency of **1** towards mammospheres grown in the presence of cobalt chloride (5 μM), a hypoxia-mimicking agent which stabilizes HIF1α, increased noticeably (Figure S22). Therefore, **1** displays selective potency towards CSCs grown in hypoxic-like conditions. Under these conditions, **1**-treatment (at the IC_{20} value, 5 days) significantly reduced the number and size of mammospheres formed (Figure S23-24).

To gain insight into the possible mechanism of action of **1** we utilized a well-established RNAi-based mechanism of action prediction methodology.[21] This methodology uses eight cancer cell lines, each harbouring a partial population of distinct GFP-tagged short hairpin RNAs (shRNAs) that target different genes involved in cell death signalling. When the cell lines are treated with a cytotoxic compound at equipotent doses, the hairpins enrich or deplete in a pattern characteristic of the mechanism of action of that compound. For new compounds, the pattern of hairpin responses (or signature) can be quantitative compared to a reference set of compounds with known mechanisms of action using a modified K-NN algorithm. Therefore we can confidently determine if a given compound belongs to a class in the reference set or a new category that is not represented in the reference set. The reference set includes all classes of clinically used cytotoxic agents, several targeted therapeutics, and some metal-based anticancer agents. Interestingly, **1** classified as a transcription/translation inhibitor (p-value = 0.05), indicating that its mechanism of action is most similar to compounds such as actinomycin D and puromycin (Figure 2C and S25). In addition to the mechanism of action classification via the K-NN algorithm, one can examine individual hairpin behaviour to glean other aspects of the mechanism of action. For instance, shChk2 enrichment is an indicator of DNA damage. In the reference set, DNA damaging agents have an average shChk2 log2(RI) value of 4.21 ± 0.69 compared to 0.36 ± 0.46 for non-DNA damaging agents. Notably, **1** has a Chk2 log2(RI) value of 0.87 ± 0.35 which is significantly higher than other non-DNA damage agents ($p < 0.05$, Mann-Whitney U-test). Taken together, this suggests that **1** is perhaps able to modify DNA in a manner that prevents transcription or translation, and this contributes to its cytotoxic effect.

Given the high nuclease activity observed for **1** in cell-free systems, the reasonable accumulation of **1** in the nucleus, and the RNAi mechanism prediction, immunoblotting studies were conducted to monitor the expression of biomarkers associated to the genomic DNA damage pathway. Specifically, the levels of the phosphorylated forms of histone H2AX (γH2AX) and Chk2 protein kinase, both of which increase due to activation of the apical kinases ATM and ATR after genomic DNA damage, were studied.[22] HMLER-shEcad cells treated with **1** (0.125-0.5 μM for 72 h) displayed a noticeable increase in the expression of γH2AX and phosphorylated Chk2, indicative of DNA damage (Figure S26). Unrepaired DNA lesions can lead to apoptosis.[23] HMLER-shEcad cells dosed with **1** (0.125-0.5 μM for 72 h) exhibited higher levels of cleaved caspase 3 and 7 compared to untreated cells (Figure S26), characteristic of caspase-dependent

apoptosis. Overall the immunoblotting studies show that **1** induces genomic DNA damage which culminates in apoptotic CSC death.

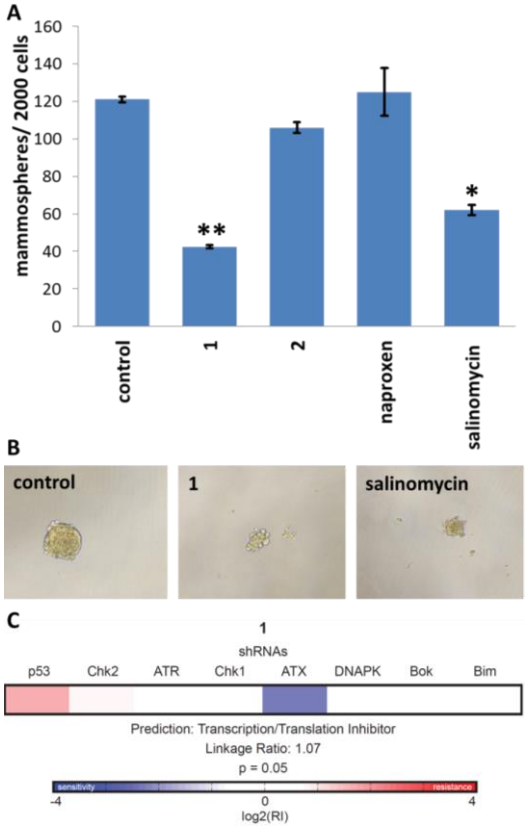


Figure 2. A) Quantification of mammosphere formation with HMLER-shEcad cells untreated and treated with **1**, **2**, naproxen, and salinomycin (at their IC_{20} values, 5 days). Error bars represent standard deviations and Student *t test*, ** = $p < 0.01$ and * = $p < 0.05$. B) Representative bright-field images (× 10) of HMLER-shEcad mammospheres in the absence and presence of **1** and salinomycin, after 5 days incubation. C) RNAi signature derived from the treatment of Eμ-Mycp$^{19arf-/-}$ lymphoma cells with **1** at the LD80−90 concentration.

Studies have found that COX-2 is aberrantly expressed in breast carcinomas.[24] Raised levels of COX-2 correlate with poor prognostic markers such as large tumor size and high tumor grade.[25] COX-2 also regulates CSCs and enhance CSC-like characteristic such as stemness, invasiveness, and tumorospheres formation.[9c,9f,26] The in vitro COX-2 inhibitory effect of **1** and naproxen was assessed using an enzyme immunoassay (EIA) (Figure S27). Upon incubation of COX-2 with **1** (0.5 μM and 5 μM), the conversion of arachidonic acid to PG was significantly reduced ($p < 0.01$, 51-64%) compared to control samples displaying 100% COX-2 activity. Naproxen inhibited COX-2 activity marginally better than **1** (the fact that **1** contains two naproxen groups per compound was taken into account). The data shows that the COX-2 inhibitory effect of naproxen is retained by **1**. Next, we investigated whether the cytotoxic mechanism of action of **1** involved COX-2 inhibition. HMLER-shEcad cells pre-treated with lipopolysaccharide (LPS) (2.5 μM for 24 h), to increase basal COX-2 levels, were treated with **1** (0.1-0.4 μM for 72 h) and the COX-2 expression was determined by immunoblotting (Figure 3A). Similar studies were also performed with **2** (20 μM for 72 h) and naproxen (20 μM for 72 h) (Figure 3A). COX-2 expression decreased in the presence of **1** in a concentration dependent manner. As expected, naproxen-treatment markedly reduced COX-2 expression. There was no observable change in COX-2 expression upon treatment with **2**. COX-2 levels were further analysed by flow cytometry using an Alexa Fluor® 488 nm labelled anti-COX-2 antibody (Figure S28). Upon treatment of HMLER-shEcad cells (pre-treated with LPS, 2.5 μM for 24 h) with **1** (0.5-2 μM for 48 h) a slight decrease in COX-2 expression was observed at the highest concentration tested. Complementary to the immunoblotting results, naproxen-treatment (20 μM for 48 h) downregulated COX-2 expression whereas **2**-treatment (20 μM for 48 h) did not. Collectively, the immunobloting and flow cytometric data suggests that the cytotoxic effect of **1** may involve COX-2 inhibition. To discern if **1** evokes COX-2-dependent CSC death, cytotoxicity studies were performed with HMLER-shEcad and HMLER cells in the presence and absence of prostaglandin E2 (PGE2) (20 μM, 72 h), the product of COX-2-mediated arachidonic acid metabolism. The potency of **1** towards HMLER-shEcad cells decreased significantly in the presence of PGE2 (4.8-fold, $p < 0.05$) (Figure 3B), suggesting that **1** induces COX-2-dependent CSC death. As the potency of **1** towards CSC-depleted, COX-2 deficient HMLER cells[13] remained largely unaltered in the presence of PGE2 (Figure 3B), the CSC-selective toxicity observed for **1** (Table 1, *vide supra*) may be due to its ability to induce CSC death through a COX-2-dependent pathway.

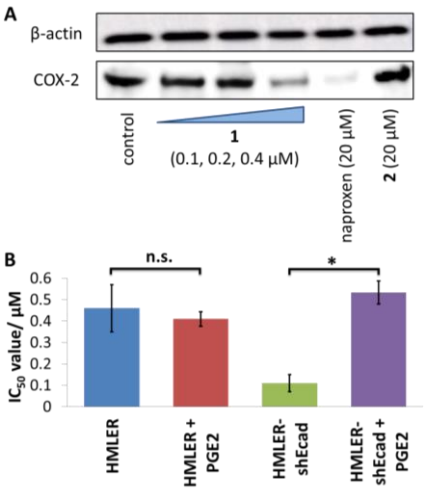


Figure 3. A) COX-2 expression in HMLER-shEcad cells pre-treated with lipopolysaccharide (LPS) (2.5 μM for 24 h), followed by treatment with **1** (0.1, 0.2, and 0.4 μM), naproxen (20 μM), or **2** (20 μM) after 72 h incubation. Whole cell lysates were resolved by SDS-PAGE and analyzed by immunoblotting against COX-2 and β-actin (loading control). B) Graphical representation of the IC_{50} values of **1** against HMLER and HMLER-shEcad cells in the absence and presence of PGE2 (20 μM). Error bars represent standard deviations and Student *t-test*, * = $p < 0.05$.

In summary, we present a cobalt(III)-cyclam complex, **1** capable of selectively killing breast CSCs over bulk breast cancer cells and reducing mammosphere formation. Mechanistic studies suggest that genomic DNA damage and COX-2 inhibition may be involved in the cytotoxic mechanism of action of **1**. Remarkably, the mammosphere-potency of **1** was enhanced in hypoxia-mimicking conditions. The findings presented in this study could pave the way for the development of new, therapeutically relevant, metal-NSAIDs conjugates that can induce CSC death through multiple pathways.

Acknowledgements ((optional))

K.S. is supported by an Early Career Fellowship (ECF-2014-178) from the Leverhulme Trust. A.E. is funded by a King's College London Faculty Graduate School International Studentship. We are grateful to Prof. Robert Weinberg for providing the cell lines used in this study.

Keywords: cobalt • cancer • antitumor agents • bioinorganic chemistry • COX inhibition

[1] L. V. Nguyen, R. Vanner, P. Dirks, C. J. Eaves, *Nat. Rev. Cancer* **2012**, *12*, 133-143.

[2] D. R. Pattabiraman, R. A. Weinberg, *Nat. Rev. Drug Discov.* **2014**, *13*, 497-512.

[3] a) M. Dean, T. Fojo, S. Bates, *Nat. Rev. Cancer* **2005**, *5*, 275-284; b) J. N. Rich, *Cancer Res.* **2007**, *67*, 8980-8984.

[4] a) K. Chen, Y.-h. Huang, J.-l. Chen, *Acta. Pharmacol. Sin.* **2013**, *34*, 732-740; b) Y. X. Feng, E. S. Sokol, C. A. Del Vecchio, S. Sanduja, J. H. Claessen, T. A. Proia, D. X. Jin, F. Reinhardt, H. L. Ploegh, Q. Wang, P. B. Gupta, *Cancer Discov.* **2014**, *4*, 702-715; c) R. Lamb, H. Harrison, J. Hulit, D. L. Smith, M. P. Lisanti, F. Sotgia, *Oncotarget* **2014**, *5*, 11029-11037.

[5] a) A. Albini, A. Bruno, C. Gallo, G. Pajardi, D. M. Noonan, K. Dallaglio, *Connect. Tissue Res.* **2015**, *56*, 414-425; b) K. Kise, Y. Kinugasa-Katayama, N. Takakura, *Adv. Drug Deliv. Rev.* **2015**.

[6] a) J. M. Heddleston, Z. Li, J. D. Lathia, S. Bao, A. B. Hjelmeland, J. N. Rich, *Br. J. Cancer* **2010**, *102*, 789-795; b) J. M. Heddleston, Z. Li, R. E. McLendon, A. B. Hjelmeland, J. N. Rich, *Cell Cycle* **2009**, *8*, 3274-3284; c) Z. Li, S. Bao, Q. Wu, H. Wang, C. Eyler, S. Sathornsumetee, Q. Shi, Y. Cao, J. Lathia, R. E. McLendon, A. B. Hjelmeland, J. N. Rich, *Cancer Cell* **2009**, *15*, 501-513; d) A. Soeda, M. Park, D. Lee, A. Mintz, A. Androutsellis-Theotokis, R. D. McKay, J. Engh, T. Iwama, T. Kunisada, A. B. Kassam, I. F. Pollack, D. M. Park, *Oncogene* **2009**, *28*, 3949-3959; e) J. Zhang, L. Li, *J. Biol. Chem.* **2008**, *283*, 9499-9503.

[7] Z. Li, J. N. Rich, *Curr. Top. Microbiol. Immunol.* **2010**, *345*, 21-30.

[8] a) K. C. Duggan, M. J. Walters, J. Musee, J. M. Harp, J. R. Kiefer, J. A. Oates, L. J. Marnett, *J. Biol. Chem.* **2010**, *285*, 34950-34959; b) O. Laneuville, D. K. Breuer, D. L. Dewitt, T. Hla, C. D. Funk, W. L. Smith, *J. Pharmacol. Exp. Ther.* **1994**, *271*, 927-934.

[9] a) B. Annabi, C. Laflamme, A. Sina, M. P. Lachambre, R. Beliveau, *Journal of neuroinflammation* **2009**, *6*, 8; b) K. H. Chen, C. C. Hsu, W. S. Song, C. S. Huang, C. C. Tsai, C. D. Kuo, H. S. Hsu, T. H. Tsai, C. Y. Tsai, L. C. Woung, S. H. Chiou, K. H. Lu, Y. W. Chen, *Childs Nerv. Syst.* **2010**, *26*, 1605-1612; c) D. Kanojia, W. Zhou, J. Zhang, C. Jie, P. K. Lo, Q. Wang, H. Chen, *Proteomics* **2012**, *12*, 3407-3415; d) K. Liao, B. Xia, Q. Y. Zhuang, M. J. Hou, Y. J. Zhang, B. Luo, Y. Qiu, Y. F. Gao, X. J. Li, H. F. Chen, W. H. Ling, C. Y. He, Y. J. Huang, Y. C. Lin, Z. N. Lin, *Theranostics* **2015**, *5*, 302-321; e) V. Sharma, D. Dixit, S. Ghosh, E. Sen, *Neurochem. Int.* **2011**, *59*, 567-571; f) B. Singh, K. R. Cook, L. Vincent, C. S. Hall, C. Martin, A. Lucci, *J. Surg. Res.* **2011**, *168*, e39-49.

[10] a) J. N. Boodram, I. J. McGregor, P. M. Bruno, P. B. Cressey, M. T. Hemann, K. Suntharalingam, *Angew. Chem. Int. Ed.* **2016**, *55*, 2845-2850; b) C. M. Moon, J. H. Kwon, J. S. Kim, S. H. Oh, K. Jin Lee, J. J. Park, S. Pil Hong, J. H. Cheon, T. I. Kim, W. H. Kim, *Int. J. Cancer* **2014**, *134*, 519-529.

[11] K. Yamada, in *Interrelations between Essential Metal Ions and Human Diseases, Vol. 13* (Eds.: A. Sigel, H. Sigel, R. K. O. Sigel), Springer Netherlands, **2013**, pp. 295-320.

[12] a) M. D. Hall, T. W. Failes, N. Yamamoto, T. W. Hambley, *Dalton Trans.* **2007**, 3983-3990; b) M. C. Heffern, N. Yamamoto, R. J. Holbrook, A. L. Eckermann, T. J. Meade, *Curr. Opin. Chem. Biol.* **2013**, *17*, 189-196; c) C. R. Munteanu, K. Suntharalingam, *Dalton Trans.* **2015**, *44*, 13796-13808.

[13] a) G. O. Ahn, K. J. Botting, A. V. Patterson, D. C. Ware, M. Tercel, W. R. Wilson, *Biochem. Pharmacol.* **2006**, *71*, 1683-1694; b) P. D. Bonnitcha, B. J. Kim, R. K. Hocking, J. K. Clegg, P. Turner, S. M. Neville, T. W. Hambley, *Dalton Trans.* **2012**, *41*, 11293-11304; c) J. Y. Chang, G. L. Lu, R. J. Stevenson, P. J. Brothers, G. R. Clark, K. J. Botting, D. M. Ferry, M. Tercel, W. R. Wilson, W. A. Denny, D. C. Ware, *Inorg. Chem.* **2013**, *52*, 7688-7698; d) J. Y.-C. Chang, R. J. Stevenson, G.-L. Lu, P. J. Brothers, G. R. Clark, W. A. Denny, D. C. Ware, *Dalton Trans.* **2010**, *39*, 11535-11550; e) T. W. Failes, C. Cullinane, C. I. Diakos, N. Yamamoto, J. G. Lyons, T. W. Hambley, *Chemistry* **2007**, *13*, 2974-2982; f) B. J. Kim, T. W. Hambley, N. S. Bryce, *Chem. Sci.* **2011**, *2*, 2135-2142; g) G. L. Lu, R. J. Stevenson, J. Y. Chang, P. J. Brothers, D. C. Ware, W. R. Wilson, W. A. Denny, M. Tercel, *Bioorg Med Chem* **2011**, *19*, 4861-4867; h) J. B. Milbank, R. J. Stevenson, D. C. Ware, J. Y. Chang, M. Tercel, G. O. Ahn, W. R. Wilson, W. A. Denny, *J. Med. Chem.* **2009**, *52*, 6822-6834; i) A. K. Renfrew, N. S. Bryce, T. Hambley, *Chemistry* **2015**, *21*, 15224-15234; j) A. K. Renfrew, N. S. Bryce, T. W. Hambley, *Chem. Sci.* **2013**, *4*, 3731-3739; k) N. Yamamoto, S. Danos, P. D. Bonnitcha, T. W. Failes, E. J. New, T. W. Hambley, *J. Biol. Inorg. Chem.* **2008**, *13*, 861-871; l) N. Yamamoto, A. K. Renfrew, B. J. Kim, N. S. Bryce, T. W. Hambley, *J. Med. Chem.* **2012**, *55*, 11013-11021.

[14] a) M. Ahmad, M. Afzal, S. Tabassum, B. Kalinska, J. Mrozinski, P. K. Bharadwaj, *Eur. J. Med. Chem.* **2014**, *74*, 683-693; b) S. S. Massoud, R. S. Perkins, F. R. Louka, W. Xu, A. Le Roux, Q. Dutercq, R. C. Fischer, F. A. Mautner, M. Handa, Y. Hiraoka, G. L. Kreft, T. Bortolotto, H. Terenzi, *Dalton Trans.* **2014**, *43*, 10086-10103; c) N. Shahabadi, S. Kashanian, F. Darabi, *Eur. J. Med. Chem.* **2010**, *45*, 4239-4245; d) W. Xu, J. A. Craft, P. R. Fontenot, M. Barens, K. D. Knierim, J. H. Albering, F. A. Mautner, S. S. Massoud, *Inorg. Chim. Acta* **2011**, *373*, 159-166.

[15] a) C. Angele-Martinez, C. Goodman, J. Brumaghim, *Metallomics* **2014**, *6*, 1358-1381; b) M. A. Husain, Z. Yaseen, S. U. Rehman, T. Sarwar, M. Tabish, *FEBS J.* **2013**, *280*, 6569-6580; c) S. Roy, S. Roy, S. Saha, R. Majumdar, R. R. Dighe, E. D. Jemmis, A. R. Chakravarty, *Dalton Trans.* **2011**, *40*, 1233-1242.

[16] P. B. Gupta, T. T. Onder, G. Jiang, K. Tao, C. Kuperwasser, R. A. Weinberg, E. S. Lander, *Cell* **2009**, *138*, 645-659.

[17] I. Morita, M. Schindler, M. K. Regier, J. C. Otto, T. Hori, D. L. DeWitt, W. L. Smith, *J. Biol. Chem.* **1995**, *270*, 10902-10908.

[18] G. Dontu, W. M. Abdallah, J. M. Foley, K. W. Jackson, M. F. Clarke, M. J. Kawamura, M. S. Wicha, *Genes Dev.* **2003**, *17*, 1253-1270.

[19] BreastCancerCare, https://www.breastcancercare.org.uk/information-support/facing-breast-cancer/going-through-treatment-breast-cancer/chemotherapy/cisplatin.

[20] T. Yi, E. Papadopoulos, P. R. Hagner, G. Wagner, *J. Biol. Chem.* **2013**, *288*, 18732-18742.

[21] a) H. Jiang, J. R. Pritchard, R. T. Williams, D. A. Lauffenburger, M. T. Hemann, *Nat. Chem. Biol.* **2011**, *7*, 92-100; b) J. R. Pritchard, P. M. Bruno, L. A. Gilbert, K. L. Capron, D. A. Lauffenburger, M. T. Hemann, *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, E170-179; c) J. R. Pritchard, P. M. Bruno, M. T. Hemann, D. A. Lauffenburger, *Mol. Biosyst.* **2013**, *9*, 1604-1619.

[22] a) J. Y. Ahn, J. K. Schwarz, H. Piwnica-Worms, C. E. Canman, *Cancer Res.* **2000**, *60*, 5934-5936; b) S. Burma, B. P. Chen, M. Murphy, A. Kurimasa, D. J. Chen, *J. Biol. Chem.* **2001**, *276*, 42462-42467; c) S. Matsuoka, G. Rotman, A. Ogawa, Y. Shiloh, K. Tamai, S. J. Elledge, *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10389-10394; d) R. Melchionna, X. B. Chen, A. Blasina, C. H. McGowan, *Nature cell biology* **2000**, *2*, 762-765; e) E. P. Rogakou, D. R. Pilch, A. H. Orr, V. S. Ivanova, W. M. Bonner, *J. Biol. Chem.* **1998**, *273*, 5858-5868.

[23] W. P. Roos, A. D. Thomas, B. Kaina, *Nat. Rev. Cancer* **2016**, *16*, 20-33.

[24] a) X. H. Liu, D. P. Rose, *Cancer Res.* **1996**, *56*, 5125-5127; b) R. A. Soslow, A. J. Dannenberg, D. Rush, B. M. Woerner, K. N. Khan, J. Masferrer, A. T. Koki, *Cancer* **2000**, *89*, 2637-2645.

[25] A. Nassar, A. Radhakrishnan, I. A. Cabrero, G. Cotsonis, C. Cohen, *Appl. Immunohistochem. Mol. Morphol.* **2007**, *15*, 255-259.

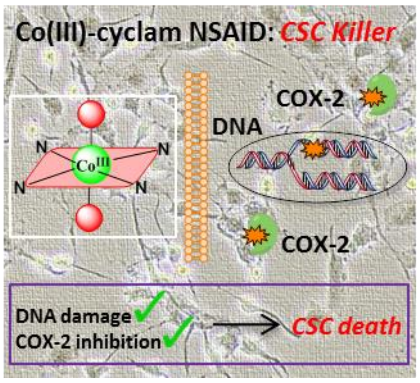
[26] B. Singh, J. A. Berry, A. Shoher, V. Ramakrishnan, A. Lucci, *Int. J. Oncol.* **2005**, *26*, 1393-1399.

Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Cancer stem cells fall for the cobait: Compounds that show cancer stem cell (CSC) specific potency are of contemporary interest given the large body of evidence linking CSCs to tumour relapse. Here we present a cobalt(III)-cyclam complex bearing two naproxen moieties that is capable of potently and selectively killing breast CSCs, both in monolayer and three-dimensional cell cultures. The complex induces its cytotoxic effect by damaging genomic DNA and inhibiting COX-2 activity.



*Author(s), Corresponding Author(s)**

Page No. – Page No.

Title